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Articles

Prorenin, Renin, Angiotensinogen, and Angiotensin-Converting Enzyme in Normal and Failing Human Hearts

Evidence for Renin Binding

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Abstract

Background A local renin-angiotensin system in the heart is often invoked to explain the beneficial effects of ACE inhibitors in heart failure. The heart, however, produces little or no renin under normal conditions.

Methods and Results We compared the cardiac tissue levels of renin-angiotensin system components in 10 potential heart donors who died of noncardiac disorders and 10 subjects with dilated cardiomyopathy (DCM) who underwent

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cardiac transplantation. Cardiac levels of renin and prorenin in DCM patients were higher than in the donors. The cardiac and plasma levels of renin in DCM were positively correlated, and extrapolation of the regression line to normal plasma levels yielded a tissue level close to that measured in the donor hearts. The cardiac tissue-to-plasma concentration (T/P) ratios for renin and prorenin were threefold the ratio for albumin, which indicates that the tissue levels were too high to be accounted for by admixture with blood and diffusion into the interstitial fluid. Cell membranes from porcine cardiac tissue bound porcine renin with high affinity. The T/P ratio for ACE, which is membrane bound, was fivefold the ratio for albumin. Cardiac angiotensinogen was lower in DCM patients than in the donors, and its T/P ratio was half that for albumin, which is compatible with substrate consumption by cardiac renin.

Conclusions These data in patients with heart failure support the concept of local angiotensin production in the heart by renin that is taken up from the circulation. Membrane binding may be part of the uptake process.

Key Words: angiotensin • renin • heart failure

Introduction

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The RAS plays an important role in the regulation of blood pressure and salt and fluid homeostasis. The kidney releases both renin and its inactive precursor prorenin into the circulation. Liver-derived angiotensinogen is cleaved in the circulating blood by renin to form Ang I, which is then converted by ACE, located on the luminal side of the vascular endothelium, into Ang II, a potent vasoconstrictor and stimulant of the release of aldosterone.

Agents that interfere with Ang II formation, the ACE inhibitors in particular, are now widely used for the treatment of hypertension and heart failure. Clinical studies indicate that the beneficial effects of ACE inhibitor treatment in heart failure and left ventricular hypertrophy are not solely determined by the effect of ACE inhibition on systemic arterial pressure.[1,2] It is, therefore, postulated that these beneficial responses are independent, at least partly, of the effects of ACE inhibition on the circulating RAS.

There is growing evidence to suggest that in cardiac tissue, Ang II is produced locally and does not originate from circulating Ang I.[3,4] However, whether the cardiac Ang I and II production depends on renin from the kidney remains a matter of dispute. In normal cardiac tissue of mice and rats, renin mRNA levels are undetectable or extremely low.[5,6] In support of this finding is the fact that after bilateral nephrectomy in the pig, cardiac renin, Ang I, and Ang II decrease to levels at or below the detection limit[4]; the same is true for cardiac Ang I

and II in the rat.[7] Renin expression, however, may be induced under pathological conditions. Renin mRNA can be detected in rat ventricle after myocardial infarction[8] and in rat atrium after low-sodium diet and treatment with the ACE inhibitor enalapril.[9]

Angiotensinogen and ACE mRNAs have been detected in normal cardiac tissue.[10] [11] [12] [13] Angiotensinogen mRNA is increased during postinfarction ventricular remodeling in the rat,[11] and ACE mRNA is increased during pressure overload–induced ventricular hypertrophy in the rat[12] and heart failure in humans.[13] Increased levels of ACE activity have been found in left ventricular aneurysms of patients after myocardial infarction.[14]

In the present study, we measured the tissue levels of prorenin, renin, angiotensinogen, and ACE in normal and failing human hearts. We compared the tissue levels of these RAS components with the levels in simultaneously obtained plasma to address the possibility of cardiac angiotensin formation independent of kidney-derived renin. The possibility of sequestration of circulating renin by cardiac tissue through binding to cardiac cell membranes was investigated in renin-binding studies with the use of cardiac membranes from freshly obtained porcine hearts.

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Collection of Cardiac Tissue and Blood Samples

Left ventricular tissue was obtained from 10 subjects (9 men and 1 woman; age, 30 to 64 years) with end-stage DCM (origin: ischemic heart disease, 7; idiopathic, 2; Becker muscular dystrophy, 1) undergoing cardiac transplantation and from 10 subjects (5 men and 5 women; age, 3 to 54 years) who had died from noncardiac causes (cerebrovascular accident, 4; polytrauma, 4; brain tumor, 2) <24 hours before the tissue arrived in the laboratory. The heart donors were not on cardiovascular medication. The medication of one subject with end-stage DCM could not be retraced. Of the remaining 9 subjects, 9 were receiving a diuretic; 8, an ACE inhibitor (captopril, 5 [average dose, 56.25 mg/d]; enalapril, 3 [average dose, 10 mg/d]); 6, a positive inotropic drug; 3, an antiarrhythmic drug; 2, isosorbide dinitrate 1, nifedipine; and 1, hydralazine.

The donor hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services Foundation/Eurotransplant Foundation) after removal of the aortic and pulmonary valves for homograft valve transplantation. The hearts had been taken out of the body immediately after circulatory arrest and maintained at 0° to 4°C in a sterile organ-protecting solution (University of Wisconsin, EuroCollins, or HTK-Bretschneider solution).[15] After arrival in the laboratory, a 5- to 10-g piece of left ventricular free wall was dissected from the heart and stored at -70°C. Pieces of left ventricular free wall (3 to 5 g) taken from the failing hearts

were frozen in liquid nitrogen and also stored at -70°C immediately after the heart had been removed from the body. Blood was obtained from 6 cardiac transplant recipients at the time of transplantation. Blood samples were collected into polystyrene tubes containing trisodium citrate (final concentration in blood, 0.013 mol/L) and centrifuged at 3000*g* for 10 minutes at room temperature. Plasma was stored at -70°C and assayed within 1 month.

Frozen cardiac tissue (≈1 g) was rapidly minced into small pieces and homogenized (1:2 [wt/vol]) in 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl, with a Polytron PT10/35 (Kinematica).[4] The cardiac tissue homogenates were stored at -20°C and assayed within 1 week.

Measurements of Renin and Prorenin

Renin in plasma was quantified by measuring Ang I generation at pH 7.4 during incubation at 37°C with a saturating concentration of sheep angiotensinogen in the presence of angiotensinase, ACE, and serine protease inhibitors (routine enzyme kinetic assay). Ang I generation under these conditions is linear for ≥3 hours, and the recovery of Ang I added to plasma before the incubation step at 37°C is 98%.[16] Due to the presence of high angiotensinase activity in cardiac tissue homogenates, Ang I generation cannot be measured by the routine enzyme kinetic assay in these homogenates, despite the addition of angiotensinase inhibitors.[4] Thus, to reliably measure Ang I–generating activity in cardiac tissue homogenates, the angiotensinases must be removed (eg, through an acidification step)[4,16] or the breakdown of Ang I must be prevented by rapidly binding it to an Ang I antibody (antibody-trapping enzyme kinetic assay).[4,17] Because acidification also leads to activation of prorenin,[4,18] we used the antibody-trapping enzyme kinetic method to measure renin in the cardiac tissue homogenates. With this assay, we found Ang I generation at 37°C to be linear for 30 minutes. In addition, the recovery of [Ile^5]Ang I added to cardiac tissue homogenates before incubation at 37°C was >75% (n=4). Results of the routine enzyme kinetic and antibody-trapping assays show good agreement.[4]

Prorenin was first converted into renin (activation) and then measured with the routine enzyme kinetic assay. Prorenin in plasma was activated with Sepharose-bound trypsin.[16] Based on our experience with the activation of prorenin in tissues,[4,19] we tested two different procedures to convert cardiac prorenin into renin (ie, acidification only or acidification followed by treatment with plasmin at neutral pH). Tissue homogenate was acidified through dialysis at 4°C for 48 hours against 0.05 mol/L glycine buffer, pH 3.3, containing 0.001 mol/L disodium EDTA and 0.095 mol/L NaCl. This was followed by (1) dialysis at 4°C for 24 hours against 0.1 mol/L phosphate buffer, pH 7.4, containing 0.001 mol/L disodium EDTA and 0.075 mol/L NaCl or (2) quick adjustment of pH to 7.4 with 1 mol/L NaOH and the subsequent addition of 0.2 vol of a solution of human plasmin (final concentration, 1 casein unit/mL) in 0.15 mol/L NaCl and incubation at 4°C for 48 hours.[4,18] The acidification

step in the two procedures effectively removed the angiotensinase activity; the recovery of [Ile^5]Ang I added to cardiac tissue homogenateat pH 7.4, after acid treatment or acid-and-plasmin treatment, was >95% in the routine enzyme kinetic assay (n=4).

Acid treatment of cardiac tissue homogenate followed by restoration of pH to 7.4 and treatment with plasmin led to virtually complete activation of prorenin, as was demonstrated by the >90% conversion and recovery of human recombinant prorenin (a gift of Dr W. Fischli, Hoffmann–La Roche, Basel, Switzerland)[4] that was added to the homogenates before the activation step (n=3). Acid treatment followed by restoration of pH to 7.4 without subsequent plasmin treatment led to less complete activation of prorenin; the recovery of added prorenin, measured as renin, was 65% to 78% (n=3). The cardiac tissue homogenates were therefore activated by the combined acid-and-plasmin method.

All samples were assessed in duplicate. Results are expressed as μU/mL or μU/g with the use of the international human kidney renin standard MRC 68/356 (Medical Research Council, National Institute of Biological Standards and Control, London, UK) as a reference. The normal range in plasma is 8 to 55 μU/mL for renin and 88 to 390 μU/mL for prorenin.[20]

Identification of Cardiac Ang I–Generating Activity as Renin

Part of the Ang I–generating activity of cardiac tissue homogenates may be related to the presence of pseudorenin (eg, cathepsin D).[21] To distinguish true renin from pseudorenin, we used the specific renin inhibitor remikiren, which has an IC_{50} value of $7x10^{-10}$ mol/L for human renin and $3.5x10^{-5}$ mol/L for bovine cathepsin D.[22] Percent inhibition of Ang I–generating activity was determined at inhibitor concentrations ranging from 10^{-11} to 10^{-5} mol/L. The inhibition curves for cardiac tissue homogenates were compared with those for plasma and the human kidney renin standard MRC 68/356.

We also used monoclonal renin antibodies to identify Ang I–generating activity as true renin. For this purpose, renin was measured with a sandwich immunoradiometric assay.[20] Monoclonal antibody R 3-36-16,[23] which reacts equally well with renin and prorenin, was biotinylated and served as a primary antibody in the assay.[24] Monoclonal antibody R 1-20-5,[25] which reacts with renin but not with prorenin, was labeled with ^{125}I (specific activity, 740 kBEq/μg) and served as the secondary antibody. The results of this assay were expressed as μU/g, with the human kidney renin standard MRC 68/356 as a reference.

Binding of Renin to Cardiac Membranes

Cardiac membranes were prepared from freshly obtained porcine left ventricular tissue (20 g) as previously described.[4] The membrane fraction contained both plasma and sarcoplasmic reticulum membranes. Semipurified kidney renin was prepared from porcine kidney.[4] Twenty-five μL of the cardiac membrane fraction, containing≈125 μg protein, was incubated in a shaking water bath at 37°C and pH 7.4 for 2 hours with 250 μL semipurified porcine

kidney renin at six different concentrations (20 to 400 pmol Ang I/min per mL). Nonspecific binding was measured by incubating renin with membranes that had been heated for 10 minutes at 95°C. The incubation was terminated by the addition of 3 mL of ice-cold 0.01 mol/L phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl, followed by rapid vacuum filtration through a Whatman GF/B filter. Filters were washed four times with 3 mL phosphate buffer and incubated with sheep angiotensinogen to measure renin according to the routine enzyme kinetic assay. The dissociation constant (K_d) and the maximum number of binding sites (B_{max}) were calculated from plots according to Scatchard.[26]

Measurements of Angiotensinogen, ACE, and Serum Albumin

Angiotensinogen was measured as the maximum quantity of Ang I that was generated during incubation at pH 7.4 and 37°C with a high concentration of human kidney renin (10 mU/mL) in the presence of inhibitors of ACE and angiotensinases.[27] The normal range in plasma is 745 to 2340 pmol/mL.[27,28]

ACE was measured with a commercial kit (ACE Color, Fujirebio; normal range, 7 to 20 mU/mL).[29]

Serum albumin was measured with single radial immunodiffusion (LC and NOR Partigen plates, Behringwerke) according to the method of Mancini et al.[30] The normal range is 22 to 43 mg/mL.[28]

Statistical Analysis

Differences between cardiac transplant recipients and donors were evaluated for statistical significance by using Student's t test or Mann-Whitney's U test for unpaired observations. Statistical significance was accepted at $P<.05$.

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Levels of Renin, Prorenin, Angiotensinogen, and ACE in Cardiac Tissue

The specific renin inhibitor remikiren, at concentrations of $\geq 10^{-8}$ mol/L, caused virtually complete inhibition of the Ang I–generating activity in the acid-and-plasmin–treated cardiac tissue homogenates (Fig 1). The inhibition curve (relating the degree of inhibition to the inhibitor concentration) for cardiac tissue homogenate treated with acid followed by plasmin was identical to the curves for untreated plasma and the human kidney renin standard, which supports the assumption that the measured Ang I–generating activity of cardiac tissue homogenate is a valid estimate of true renin. The IC_{50} value was $\approx 3 \times 10^{-10}$ mol/L, which is in accordance with reports in the literature.[22] The inhibition curve for untreated cardiac tissue

homogenate differed somewhat from the inhibition curves for plasma renin and kidney renin (Fig 1⊡); the difference can be accounted for by assuming that 30% of the Ang I–generating activity in untreated cardiac homogenate was caused by pseudorenin. We therefore measured true renin in untreated cardiac tissue homogenates as the difference between the Ang I–generating activity in the absence of remikiren and the Ang I–generating activity in the presence of 10^{-5} mol/L remikiren.

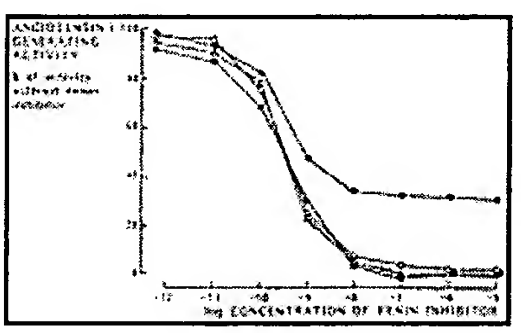

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Figure 1. Inhibition of angiotensin I–generating activity by increasing concentrations of the renin inhibitor remikiren. (•), Untreated cardiac tissue homogenate; (○), cardiac tissue homogenate pretreated with acid and plasmin; (△), human kidney renin standard MRC 68/356; and (■), plasma.

Comparison between the results of the enzyme kinetic and immunoradiometric assays of renin in cardiac tissue homogenate and plasma from 5 subjects with DCM showed good agreement (Fig 2⊡), confirming that the enzyme kinetic assay is indeed a valid measurement of true renin. According to the enzyme kinetic assay, the plasma concentration of naturally occurring renin in subjects with DCM was 80±23% (mean±SD) of the renin concentration after prorenin activation compared with 65±33% according to the immunoradiometric assay (difference not significant). The cardiac tissue concentration of naturally occurring renin in these subjects was 59±19% of the renin concentration after prorenin activation in the enzyme kinetic assay and 77±11% in the immunoradiometric assay (difference not significant).

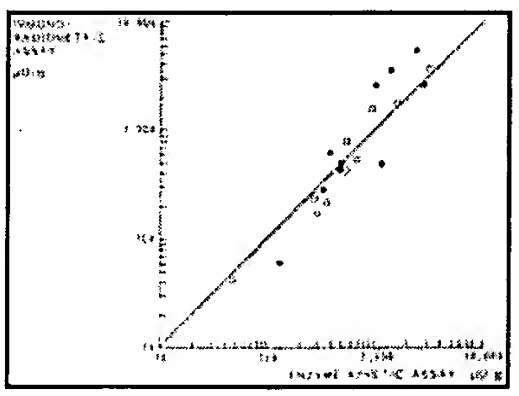

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Figure 2. Renin measured by enzyme kinetic assay versus renin measured by immunoradiometric assay in cardiac tissue (• and ○) and plasma (■ and □) before (○ and □) and after (• and ■) prorenin activation (r=.92, P<.01).

Fig 3 is a comparison of the cardiac tissue levels of renin, prorenin, angiotensinogen, ACE, and serum albumin between the DCM patients and the donors. The levels of renin and prorenin were more than fivefold higher in the patients, whereas angiotensinogen was lower. Cardiac ACE was not different between the two groups. The cardiac tissue level of serum albumin was somewhat higher in the DCM patients than in the donors.

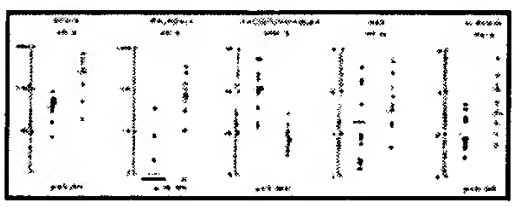

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Figure 3. Renin, prorenin, angiotensinogen, ACE, and albumin in cardiac tissue of heart donors (•) and patients with DCM (○). Prorenin was below the detection limit in 7 donors and 1 patient. Median (renin and prorenin) and mean (angiotensinogen, ACE, and albumin) values are shown. Differences between donors and patients were tested with Mann-Whitney's *U* test (renin and prorenin) or Student's *t* test (angiotensinogen, ACE, and albumin) for unpaired observations.

Plasma levels at the time of heart transplantation were available for 6 of the 10 DCM cases. Plasma renin in these subjects ranged from 472 to 3028 μU/mL, which is more than sevenfold the normal level. Plasma prorenin ranged from 180 to 1214 μU/mL, and in 3 subjects it was above normal. Plasma angiotensinogen ranged from 77 to 484 pmol/mL, which is below the normal range. Plasma ACE ranged from 5.8 to 28.9 mU/mL, and in 3 subjects it was above normal. Albumin in plasma was normal; it ranged from 22 to 34 mg/mL.

The cardiac tissue-to-plasma concentration ratio for serum albumin was≈12% (Fig 4), which is in agreement with the fact that the localization of this protein is restricted to the extracellular fluid. Albumin (molecular mass, 70 kD) is known to be present in the interstitial fluid, albeit in lower concentrations than in plasma.[31] The cardiac tissue-to-plasma concentration ratio for angiotensinogen (molecular mass, 65 kD) was 6%, which is in keeping with the contention that cardiac angiotensinogen is also localized in the extracellular fluid. The lower ratio for angiotensinogen than for albumin may suggest that the angiotensinogen consumption rate, and therefore the Ang I production rate, is higher in cardiac tissue than in circulating plasma. The cardiac tissue levels of renin and angiotensinogen were negatively correlated (Fig 5), most likely because increased renin leads to increasedsubstrate consumption. The cardiac tissue-to-plasma ratio for ACE was 54%, which was much higher than the ratio for serum albumin. Most of the ACE in cardiac tissue is probably cell membrane bound.[4] The cardiac tissue-to-plasma concentration ratios for renin and prorenin (molecular mass, 48 and 54 kD, respectively) were 30% and 36%, which was also higher than the ratio for serum albumin. This suggests that the localization of these proteins is not restricted to the extracellular fluid compartment.

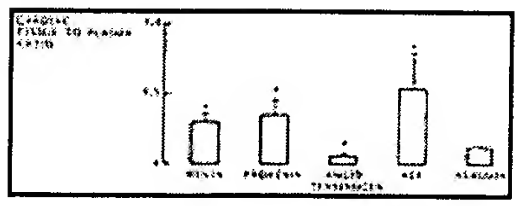

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Figure 4. Cardiac tissue-to-plasma concentration ratios for renin, prorenin, angiotensinogen, ACE, and albumin in patients with DCM. Data are mean±SD. *P<.005 vs albumin; #P<.05 vs albumin (Student's t test for paired observations).

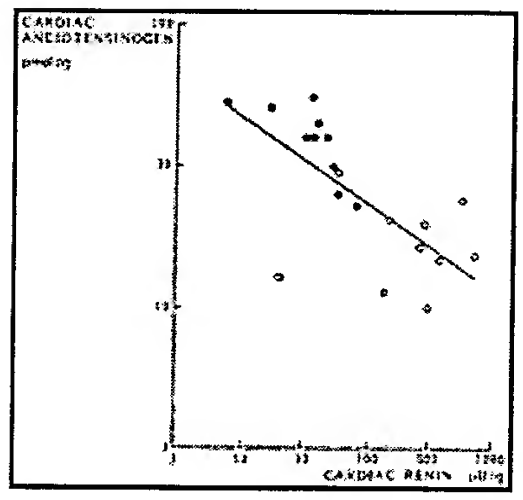

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Figure 5. Relationship between cardiac renin and cardiac angiotensinogen in heart donors (•) and patients with DCM (○) (r=.72, P<.05).

The cardiac tissue levels of renin in the subjects with DCM were directly correlated with the plasma levels of renin (log [cardiac renin]=0.50xlog [plasma renin]+0.93; r=.84, P<.05). There was no significant correlation between the prorenin levels in cardiac tissue and plasma.

Binding of Renin to Cardiac Membranes

Cardiac membranes prepared from porcine left ventricular tissue (n=8) bound porcine renin in a dose-dependent way (Fig 6). Binding was maximal within 15 minutes. According to Scatchard analysis, K_d was 0.21±0.11 nmol Ang I/min per mL and B_{max} was 0.5±0.3 pmol Ang I/min per mg protein. One nanomole of pure porcine renin generates during incubation at 37°C with saturating concentrations of porcine angiotensinogen≈200 nmol Ang I/min.[32,33] With our semipurified preparation of porcine kidney renin, we found the maximum reaction velocity (V_{max}) when incubated with sheep angiotensinogen to be similar to the V_{max} when incubated with angiotensinogen prepared from nephrectomized pigs.[34] Thus, K_d was $\approx 10^{-9}$ mol/L, and B_{max} was≈2 fmol/mg protein.

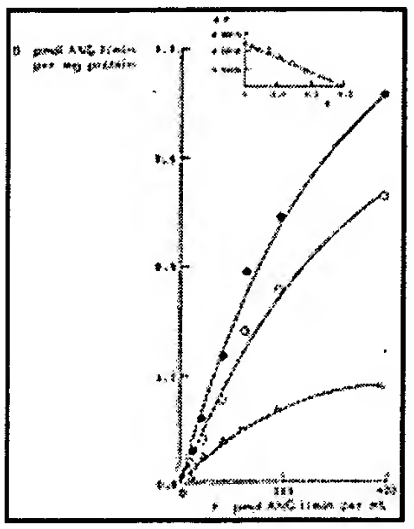

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Figure 6. Binding of semipurified porcine kidney renin to cardiac membranes prepared from porcine left ventricular tissue. Specific binding (△) was taken as the difference between total binding (•) during incubation of renin with intact cardiac membranes and nonspecific binding (○) during incubation of renin with cardiac membranes after their denaturation by heating. K_d and B_{max} values were calculated from a plot according to Scatchard[26] (inset).

Discussion

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The results of the present study demonstrate the presence of increased concentrations of renin and prorenin in left ventricular tissue from patients with DCM. The cardiac levels of renin and prorenin were more than fivefold the cardiac levels in the donors. In addition, the cardiac tissue-to-plasma concentration ratios for renin and prorenin (molecular mass, 48 and 54 kD, respectively) were approximately threefold the ratio for serum albumin (molecular mass, 70 kD), indicating that the levels of renin and prorenin in cardiac tissue were too high to be explained by admixture with blood or by diffusion from the blood into the interstitial fluid. In normal porcine left ventricular tissue, the renin level was also higher than can be explained by its localization in extracellular fluid.[4]

Purified membrane fractions prepared from porcine left ventricular tissue contained renin,[4] and specific binding of renin and prorenin to rat renal and other tissue membranes has been reported.[35] [36] In the present study, we observed high-affinity binding of porcine renin to porcine cardiac membranes. Because the membrane fraction contained both plasma membranes and sarcoplasmic reticulum, it is unclear whether renin binding was specific for plasma membranes. Chemical cross-linking studies also suggest the presence of renin-binding proteins in rat vascular tissue membranes.[37] Our findings in the human heart, which indicate that renin and prorenin in cardiac tissue are not restricted to the extracellular fluid, may therefore be explained by binding to cell membranes.

ACE, which is known to be a membrane-bound enzyme, was also found in cardiac tissue in concentrations that were higher than could be explained by its presence in the extracellular fluid. Cardiac ACE levels did not differ between the DCM patients and the donors. It should

be noted, however, that 8 of the 10 patients with DCM were receiving ACE inhibitor treatment at the time of cardiac transplantation. This may have led to some underestimation of ACE activity in these patients. In normal hearts, ACE appears to be limited to the vascular endothelium and the endocardium.[38] [39] After myocardial infarction in humans, ACE can also be detected in the remaining viable cardiomyocytes near the infarct scar of the aneurysmal left ventricle,[38] as well as in fibroblasts, vascular smooth muscle cells, and macrophages in the scar area itself.[38] After coronary occlusion in rats, ACE was demonstrated in fibroblasts in the healthy hypertrophying part of the heart.[40]

The patients with DCM had markedly increased levels of renin and reduced levels of angiotensinogen in circulating plasma at the time of transplantation. These are characteristic findings in patients with end-stage heart failure who are treated with diuretics and ACE inhibitors.[41] [42] The cardiac level of angiotensinogen in these patients was approximately one third of the level in the donors and was negatively correlated with the renin concentration. This is probably related to the fact that substrate consumption is increased when renin is increased. An interesting finding was that the cardiac tissue-to-plasma concentration ratio for angiotensinogen (molecular mass, 65 kD) was half the ratio for albumin (molecular mass, 70 kD). This is evidence in favor of consumption of angiotensinogen that is present in the cardiac extracellular fluid through the reaction with renin, thereby implying that local Ang I production occurs in the heart.

In the DCM patients in whom measurements of renin were available in cardiac tissue as well as in plasma, the tissue and plasma levels showed a positive correlation. Extrapolation of the regression line to normal plasma concentrations yielded a tissue concentration of renin close to the concentration measured in the donor hearts. Thus, there was no evidence to suggest that the regulation of cardiac renin is independent of the regulation of circulating renin. A previous study by our group of the effect of nephrectomy on the cardiac levels of renin in healthy pigs showed that most, if not all, renin in cardiac tissue originates from the kidney.[4] Studies in which intravenous bolus injections of radiolabeled renin were given to rats and monkeys have demonstrated that the enzyme accumulated in the heart.[43] [44]

Prorenin in cardiac tissue was not significantly correlated with prorenin in plasma. It should be noted, however, that the measurement of prorenin is not as accurate as the measurement of renin. Renin was measured as the Ang I–generating activity without prior in vitro activation of prorenin, whereas prorenin was measured as the difference between the Ang I–generating activity after prorenin activation and the Ang I–generating activity before activation. The results of this subtraction method are sufficiently accurate when renin is low compared with prorenin, as is the case in normal plasma. Results are less accurate when the renin-to-prorenin ratio is higher, as was the case in both plasma and cardiac tissue in the patients with DCM. Another explanation for the apparent lack of a correlation between the measured levels of

prorenin in cardiac tissue and plasma might be that part of the plasma-derived prorenin is activated in cardiac tissue. Such in vivo activation of plasma-derived prorenin in extrarenal tissues, however, has never been demonstrated.

In addition to the evidence presented here, there is growing evidence from the literature to support the existence of local angiotensin formation in cardiac tissue. Perfusion of the isolated rat heart with renin leads to Ang I and II release into the coronary effluent[3] and to Ang I release into the interstitial fluid.[45] Without the addition of renin to the perfusion fluid, the cardiac release of Ang I and II was practically zero. The study reported here provides no data on Ang I and II levels in cardiac tissue and plasma. Special precautions are required when samples for measuring these peptides are collected, and these precautions could not always be taken in the setting of this study. It appears logical to assume that the elevated renin concentrations in the hearts affected by DCM will promote cardiac Ang I and II production. Experiments in intact pigs showing reduced cardiac contractility in response to intracoronary administration of the renin inhibitor remikiren support the fact that cardiac Ang II production has functional significance[46] ; the time course of this response was not correlated with the effects of the inhibitor on the levels of Ang I and II in the circulation. Intracardiac Ang I and II production may also participate in the long-term processes of hypertrophy and remodeling after myocardial infarction.[47]

In summary, our results add to the growing evidence in support of local Ang I and II production by the heart. So far, attempts to show that the production of these peptides is independent of renin from the circulation have failed, at least in normal hearts. The present study also fails to provide such evidence in DCM patients with severe heart failure; rather, our results are in keeping with the contention that renin from the circulation is taken up by the heart and that tissue binding of renin is part of the uptake process.

Selected Abbreviations and Acronyms

Ang	= angiotensin
DCM	= dilated cardiomyopathy
RAS	= renin-angiotensin system

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